PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ÉTATS-UNIS D'AMÉRIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 03 November 1999 (03.11.99)	
International application No. PCT/SE99/00452	**Applicant's or agent's file reference** 2998173
International filing date (day/month/year) 23 March 1999 (23.03.99)	**Priority date** (day/month/year) 26 March 1998 (26.03.98)
Applicant JAREKRANS, Mats	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

14 September 1999 (14.09.99)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer

Claudio Borton

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PATENT COOPERATION TREATY

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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference PC-2998173	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/SE99/00452	International filing date *(day/month/year)* 23.03.1999	Priority date *(day/month/year)* 26.03.1998
International Patent Classification (IPC) or national classification and IPC7 C12N 5/08		
Applicant Bionative AB et al		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 4 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 3 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
14.09.1999	07.06.2000
Name and mailing address of the IPEA/SE Patent- och registreringsverket Box 5055 S-102 42 STOCKHOLM Telex 17978 PATOREG-S Facsimile No. 08-667 72 88	Authorized officer Carl-Olof Gustafsson / MRo Telephone No. 08-782 25 00

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

[redacted]tional application No.
PCT/SE99/00452

I. Basis of the report

1. This report has been drawn on the basis of *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.)*:

☐ the international application as originally filed.

☒ the description, pages 1-16, as originally filed,
pages _____, filed with the demand,
pages _____, filed with the letter of _____,
pages _____, filed with the letter of _____.

☒ the claims, Nos. _____, as originally filed,
Nos. _____, as amended under Article 19,
Nos. _____, filed with the demand,
Nos. 1-19, filed with the letter of 19.04.2000,
Nos. _____, filed with the letter of _____.

☒ the drawings, ~~sheets~~/fig 1-2, as originally filed,
~~sheets~~/fig _____, filed with the demand
~~sheets~~/fig _____, filed with the letter of _____,
~~sheets~~/fig _____, filed with the letter of _____.

2. The amendments have resulted in the cancellation of:

☐ the description, pages _____
☐ the claims, Nos. _____
☐ the drawings, ~~sheets~~/fig _____

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the supplemental Box (Rule 70.2(c)).

4. Additional observations, if necessary:

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/SE99/00452

V. Resoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	1-19	YES
	Claims		NO
Inventive step (IS)	Claims	1-19	YES
	Claims		NO
Industrial applicability (IA)	Claims	1-19	YES
	Claims		NO

2. Citations and explanations

Reasoned statement

The present application discloses a process and apparatus for the continuous purification of blood. The process comprises the following steps:
- blood plasma is being separated from the leukocyte solution
- ammonium chloride is added to the filtered leukocyte solution
- mixing
- centrifugation
- the harvested cells are collected for further treatment

The International Search Report revealed two documents that are considered to be of particular relevance:

A. US4294824 (see entire document)
B. US4938876 (see especially column 3, lines 26-28; column 7, lines 24-45)

Document A discloses a method in which in a first step blood cells are being lysed, for instance using ammonium chloride. In a second step centrifugation is performed, followed by a filtration step. The method disclosed in the present application differs from the method of document A essentially concerning the order of performing the different separation steps. Referring to the applicant's reasoned statement in the priority application, it is not considered obvious to a person skilled in the art to modify the order of performing the unit operations disclosed in document A. Particular advantages are achieved using the process of the present application. Claims 1-11 are thus considered to fulfil the requirements of novelty and inventive step.

.../...

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: V.

Document B discloses an apparatus consisting of a membrane filter, a static mixer, a retention vessel and a centrifuge for continuous purification of leukocytes. However, the different parts of the apparatus are connected differently to each other from what is disclosed in claim 12. The apparatus disclosed in claim 12 is adapted for performing the continuous purification according to the method disclosed in claims 1-11. Thus, claims 12-19 are considered to fulfil the requirement of novelty and inventive step.

The invention as disclosed in claims 1-19 is considered to be industrially applicable.

CLAIMS

1. Process for the continuous purification and concentration of leukocytes from blood, characterized in that said process comprises the following steps:

(a) separating plasma from the blood by filtration in order to achieve a filtered buffy coat fraction;

(b) adding an aqueous solution, which is hypotonic in relation to plasma, to the buffy coat fraction from step (a), in order to achieve lysation of erythrocytes contained in the buffy coat fraction;

(c) mixing the buffy coat fraction and the aqueous hypotonic solution from step (b) in a mixing device;

(d) leading the mixture from step (c) through a retention vessel;

(e) leading the mixture from step (d) through a centrifuge in order to separate the leukocytes ;

(f) collecting the separated leukocytes from step (e).

2. Process according to claim 1, characterized in that a buffy coat fraction, obtained from blood, is used in stead of blood in step (a) and plasma is removed from this buffy coat fraction by filtration.

3. Process according to claim 1 or 2, characterized in that in step (b) the aqueous hypotonic solution is ammonium chloride.

4. Process according to any of the claims 1 - 3, characterized in that the filtration is performed by leading the blood through a membrane filter with a pore size in the interval of 0.1 - 1.0 µm.

5. Process according to claim 4, characterized in that the filtration is performed by leading the blood through a membrane filter with a pore size in the interval of 0.4 - 0.6 µm.

6. Process according to any of the claims 1 - 5, characterized in that the retention vessel is designed in a way resulting in a retention time for the mixture in step (d) of about 0.5 - 10 minutes.

7. Process according to any of the claims 1 - 6, characterized in that the leukocytes collected in step (f) are subjected to a second lysis step.

8. Process according to claim any of the claims 1 - 7, characterized in that the leukocytes collected in step (f) are incubated in a bioreactor for interferon production.

9. Process according to claim 1 or 2, characterized in that the plasma separated in step (a) is recovered.

10. Process according to any of the claims 1 - 7, characterized in that the process is automatically operated and adapted for clean in place (CIP) cleaning and (SIP) sanitation in place.

11. Process according to any of the preceding claims, characterized in that the blood is human blood.

12. Apparatus for continuous purification and concentration of leukocytes, from blood, characterized in that said apparatus includes the following means:

(i) a membrane filter means for separating plasma from the blood by filtration in order to achieve a filtered buffy coat fraction;

(ii) a static mixer means connected to said membrane filter means and receiving said buffy coat fraction for mixing the buffy coat fraction and an aqueous hypotonic solution in order to achieve lysation of erythrocytes contained in the buffy coat fraction of the mixture;

(iii) a retention vessel means, connected to said static mixer means for receiving the mixture therefrom and designed in a way for the mixture to become homogeneous;

(iv) a centrifuge means connected to said retention vessel means and arranged to separate the leukocytes from the mixture from the retention vessel.

13. Apparatus according to claim 12, characterized in that, a buffy coat fraction, obtained from blood, is used in stead of blood and plasma is removed from this buffy coat fraction by filtration.

14. Apparatus according to claim 12, characterized in that the aqueous hypotonic solution is ammonium chloride.

15. Apparatus according to claim 12, characterized in that the membrane filter means is a filter with a pore size in the interval of 0.1 - 1.0 µm.

16. Apparatus according to claim 14, characterized in that the membrane filter means is a filter with a pore size in the interval of 0.4 - 0.6 µm.

17. Apparatus according to claim 12, characterized in that the retention vessel means is designed in a way resulting in a retention time for the mixture in the retention vessel of about 0.5 - 10 minutes.

18. Apparatus according to claim 12, characterized in that the centrifuge is adapted to continuous or semi-continuous separation of the leukocytes.

19. Apparatus according to claim 12, characterized in that said apparatus is equipped for cleaning and sanitation, which cleaning and sanitation does not require the dismantling of the equipment, so called clean in place (CIP) and sanitation in place (SIP).

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International Bureau

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(51) International Patent Classification 6 :
C12N 5/08

A1

(11) International Publication Number: WO 99/49016

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(30) Priority Data:

9801029-1	26 March 1998 (26.03.98)	SE
60/085,391	14 May 1998 (14.05.98)	US

(63) Related by Continuation (CON) or Continuation-in-Part (CIP) to Earlier Application
US 60/085,391 (CON)
Filed on 14 May 1998 (14.05.98)

(71) Applicant *(for all designated States except US)*: BIONATIVE AB [SE/SE]; Tvistevägen 48, S–907 36 Umeå (SE).

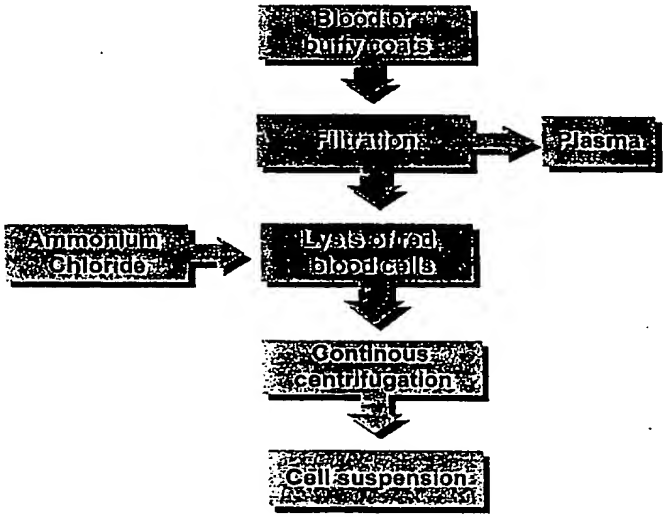
(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: JAREKRANS, Mats [SE/SE]; Trastvägen 19 B, S–906 54 Umeå (SE).

(74) Agent: AWAPATENT AB; P.O. Box 45086, S–104 30 Stockholm (SE).

(81) Designated States: AE, AL, AM, AT, AT (Utility model), AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, CZ (Utility model), DE, DE (Utility model), DK, DK (Utility model), EE, EE (Utility model), ES, FI, FI (Utility model), GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK (Utility model), SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: PROCESS FOR CONTINUOUS PURIFICATION AND CONCENTRATION OF LEUKOCYTES FROM BLOOD



(57) Abstract

Process and apparatus for the continuous purification and concentration of leukocytes from blood, characterized in that said process comprises the following steps: (a) separating plasma from the blood by filtration in order to achieve a filtered buffy coat fraction; (b) adding an aqueous solution, which is hypotonic in relation to plasma, to the buffy coat fraction from step (a), in order to achieve lysation of erythrocytes contained in the buffy coat fraction; (c) mixing the buffy coat fraction and the aqueous hypotonic solution from step (b) in a mixing device; (d) leading the mixture from step (c) through a retention vessel; (e) leading the mixture from step (d) through a centrifuge in order to separate the leukocytes; (f) collecting the separated leukocytes from step (e).

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 99/00452

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: C12N 5/08
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: A61K, C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 4294824 A (WILLIAM A. JONES ET AL), 13 October 1981 (13.10.81) --	1-19
A	US 4938876 A (ERNEST O. OHSOL), 3 July 1990 (03.07.90), See column 3, lines 26-28; column 7, lines 24-26 -- --------	12-19

☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" erlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
29 June 1999	23 -07- 1999
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Carl-Olof Gustafsson/Els Telephone No. +46 8 782 25 00

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Information on patent family members

01/06/99

International application No.
PCT/SE 99/00452

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 4294824 A	13/10/81	AR 211931 A	14/04/78
		AU 515950 B	14/05/81
		AU 1661476 A	09/02/78
		BE 844875 A	04/02/77
		CA 1063019 A	25/09/79
		DE 2635065 A,C	17/02/77
		DK 350876 A	06/02/77
		FI 762178 A	06/02/77
		FR 2320106 A,B	04/03/77
		GB 1579120 A	12/11/80
		IE 44378 B	04/11/81
		IN 144537 A	13/05/78
		JP 52018807 A	12/02/77
		LU 75535 A	13/02/78
		NL 7608651 A	08/02/77
		NZ 181653 A	11/01/79
		SE 7608771 A	06/02/77
		ZA 7604643 A	29/03/78
US 4938876 A	03/07/90	CA 2048635 A,C	03/09/90
		DE 69012988 D,T	11/05/95
		EP 0465485 A,B	15/01/92
		ES 2064724 T	01/02/95
		JP 5504714 T	22/07/93
		WO 9009833 A	07/09/90

CLAIMS

1. Process for the continuous purification and concentration of leukocytes from blood, characterized in that said process comprises the following steps:

(a) separating plasma from the blood by filtration in order to achieve a filtered buffy coat fraction;

(b) adding an aqueous solution, which is hypotonic in relation to plasma, to the buffy coat fraction from step (a), in order to achieve lysation of erythrocytes contained in the buffy coat fraction;

(c) mixing the buffy coat fraction and the aqueous hypotonic solution from step (b) in a mixing device;

(d) leading the mixture from step (c) through a retention vessel;

(e) leading the mixture from step (d) through a centrifuge in order to separate the leukocytes ;

(f) collecting the separated leukocytes from step (e).

2. Process according to claim 1, characterized in that a buffy coat fraction, obtained from blood, is used in stead of blood in step (a) and plasma is removed from this buffy coat fraction by filtration.

3. Process according to claim 1 or 2, characterized in that in step (b) the aqueous hypotonic solution is ammonium chloride.

4. Process according to any of the claims 1 - 3, characterized in that the filtration is performed by leading the blood through a membrane filter with a pore size in the interval of 0.1 - 1.0 μm.

5. Process according to claim 4, characterized in that the filtration is performed by leading the blood through a membrane filter with a pore size in the interval of 0.4 - 0.6 μm.

6. Process according to any of the claims 1 - 5, characterized in that the retention vessel is designed in a way resulting in a retention time for the mixture in step (d) of about 0.5 - 10 minutes.

7. Process according to any of the claims 1 - 6, characterized in that the leukocytes collected in step (f) are subjected to a second lysis step.

8. Process according to claim any of the claims 1 - 7, characterized in that the leukocytes collected in step (f) are incubated in a bioreactor for interferon production.

9. Process according to claim 1 or 2, characterized in that the plasma separated in step (a) is recovered.

10. Process according to any of the claims 1 - 7, characterized in that the process is automatically operated and adapted for clean in place (CIP) cleaning and (SIP) sanitation in place.

11. Process according to any of the preceding claims, characterized in that the blood is human blood.

12. Apparatus for continuous purification and concentration of leukocytes, from blood, characterized in that said apparatus includes the following means:

(i) a membrane filter means for separating plasma from the blood by filtration in order to achieve a filtered buffy coat fraction;

(ii) a static mixer means for mixing the buffy coat fraction and an aqueous hypotonic solution in order to achieve lysation of erythrocytes contained in the buffy coat fraction;

(iii) a retention vessel means;

(iv) a centrifuge means in order to separate the leukocytes.

13. Apparatus according to claim 12, characterized in that, a buffy coat fraction, obtained from blood, is used in stead of blood in (a) and plasma is removed from this buffy coat fraction by filtration.

14. Apparatus according to claim 12, characterized in that in (b) the aqueous hypotonic solution is ammonium chloride.

15. Apparatus according to claim 12, characterized in that the membrane filter means is a filter with a pore size in the interval of 0.1 - 1.0 μm.

16. Apparatus according to claim 14, characterized in that the membrane filter means is a filter with a pore size in the interval of 0.4 - 0.6 μm.

17. Apparatus according to claim 12, characterized in that the retention vessel means is designed in a way resulting in a retention time for the mixture in the retention vessel of about 0.5 - 10 minutes.

18. Apparatus according to claim 11, characterized in that the centrifuge is adapted to continuous or semi-continuous separation of the leukocytes.

19. Apparatus according to claim 11, characterized in that said apparatus is equipped for cleaning and sanitation, which cleaning and sanitation does not require the dismantling of the equipment, so called clean in place (CIP) and sanitation in place (SIP).